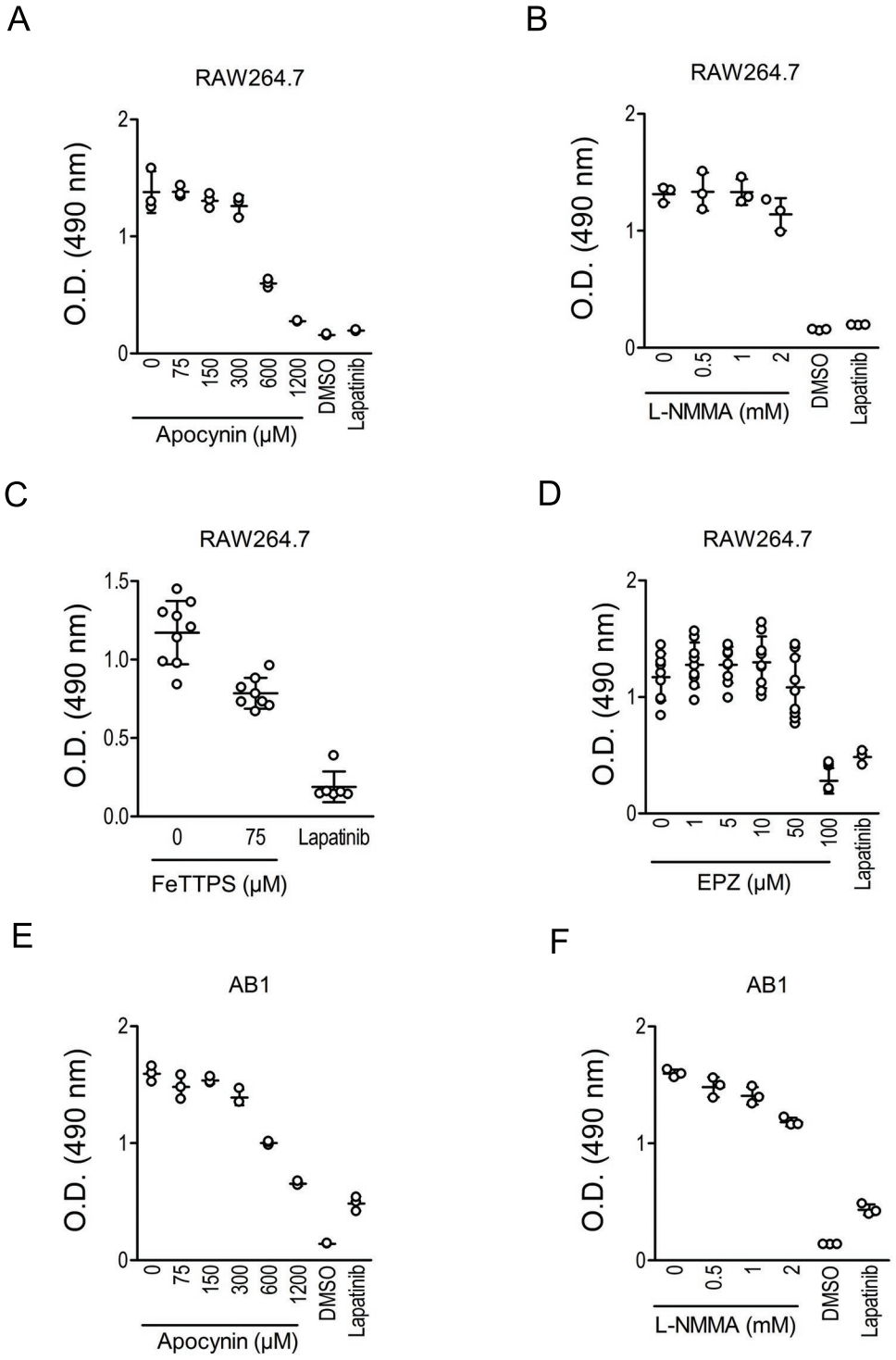

Figure S1: Dose response of L-NMMA, apocynin, FeTTPS and EPZ on metabolic activity of RAW267.4 and AB1 cells

RAW264.7 **(A)** and AB1 **(B)** cells were cultivated in a 96-well plate and treated with different concentrations apocynin (0-1200 μM), L-NMMA (0-2 mM), FeTTPS (75μM) and EPZ-5687 (0-100 μM). The cell viability was evaluated after 48 hours incubation using the MTS assay (Promega). After incubation at 37°C in presence of 20 μL tetrazolium-containing reagent, the 96-well plate was analyzed using a colorimetric microplate reader at a wavelength of 490 nm (Victor3V 1420 multilabel counter; Perkin Elmer Wallac). Y-axis represents optical densities (O.D.).

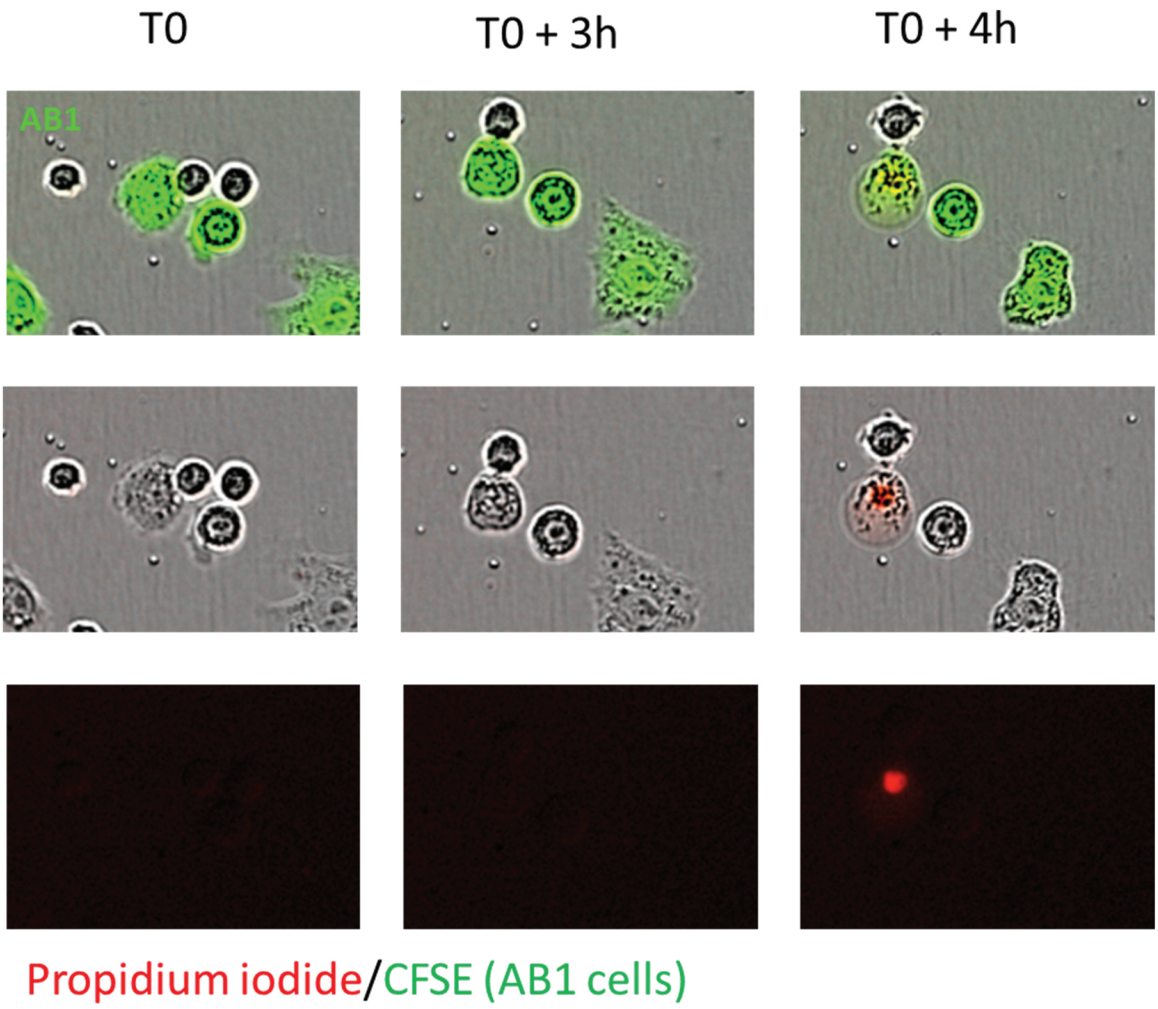

Figure S2: Direct cytotoxicity of RAW264.7 macrophages in contact with AB1 mesothelioma cells

RAW264.7 macrophages were co-cultivated during 24 h with CFSE-labeled AB1 cells at a 1:1 ratio in presence of 0.5µM propidium iodide (PI). PI incorporation into AB1 cells was recorded by time-lapse microscopy using the IncuCyte S3 Live-Cell imaging system (Essen Bioscience) in an incubator maintained at 37°C in a humidified 5% CO_2 atmosphere.

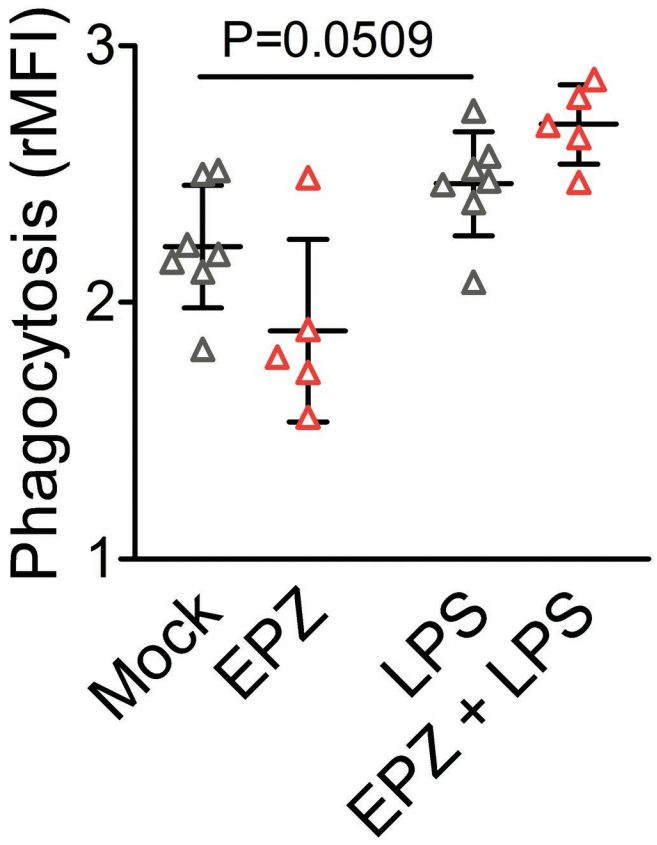

Figure S3: EPZ does not affect dextran phagocytosis by RAW267.4

RAW264.7 macrophages were incubated with 100 ng/ml of fluorescein isothiocyanate (FITC) dextran (Sigma-Aldrich) for 30 min at 37°C and washed in ice-cold PBS. After an additional wash with PBS, cell uptake of FITC-dextran was measured at 525 nm using a FACS-Aria cytometer and analyzed with the FACS Diva software (Becton Dickinson). Emission data were expressed in fold increase of the mean intensity of fluorescence (MFI) of FITC-dextran measured at 37°C versus control (MFI of untreated cells). Statistical significance was evaluated using one-way ANOVA.

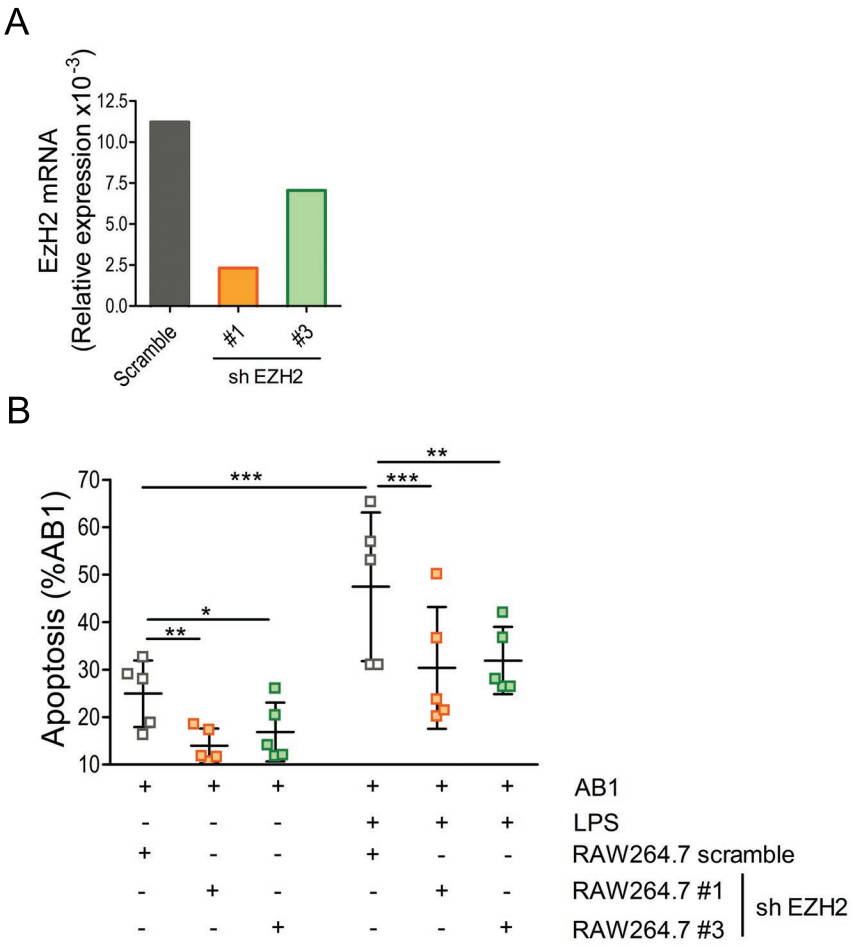

Figure S4: Genetic inhibition of EZH2 reduces direct cytotoxicity of RAW264.7 macrophages

RAW264.7 macrophages were transduced with lentiviral vectors co-expressing GFP and shRNA sequences targeting EZH2 (shRNA#1, shRNA#3) or mock (scramble) (Vector Builder). **(A)** Total RNA was prepared from whole cells using NucleoSpin RNA Plus Kit (Macherey-Nagel) according manufacturer's instructions. After reverse transcription using FastGene Scriptase II cDNA Kit (Nippon Genetics), the abundance of transcripts was assessed by real-time qPCR analysis using the Takyon™ SYBR MasterMix (Eurogentec) and primers specific for EZH2 (5'-CAGATAAGGGCACCGCAGAA-3' ; 5'- ACATTCAGGAGGCAGAGCAC-3') and GAPDH (5'-CGGAGTCAACGGATTTGGTCGTAT-3' ; 5'- AGCCTTCTCCATGGTGGTGAAGAC-3'). Samples were amplified in triplicate on each plate and data were analyzed using LightCycler 480 Software (Roche Diagnostics). Normalization was performed using mouse GAPDH as an internal control, and relative gene expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ method. **(B)** The different RAW264.7 cell lines were cultivated in presence or absence of LPS during 24 hours. After 3 washes in PBS, RAW264.7 macrophages were co-cultivated with AB1 cells at a 10:1 ratio during 48 hours. Apoptotic rates of AB1 cells were determined by flow cytometry after staining with the Annexin V-FITC kit (Becton Dickinson). Mean values and standard deviations result from six independent experiments. Each bar represents the mean +/- SEM from 5 independent experiments. Statistical significance was evaluated using one-way ANOVA followed by Tukey's multiple comparison test (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

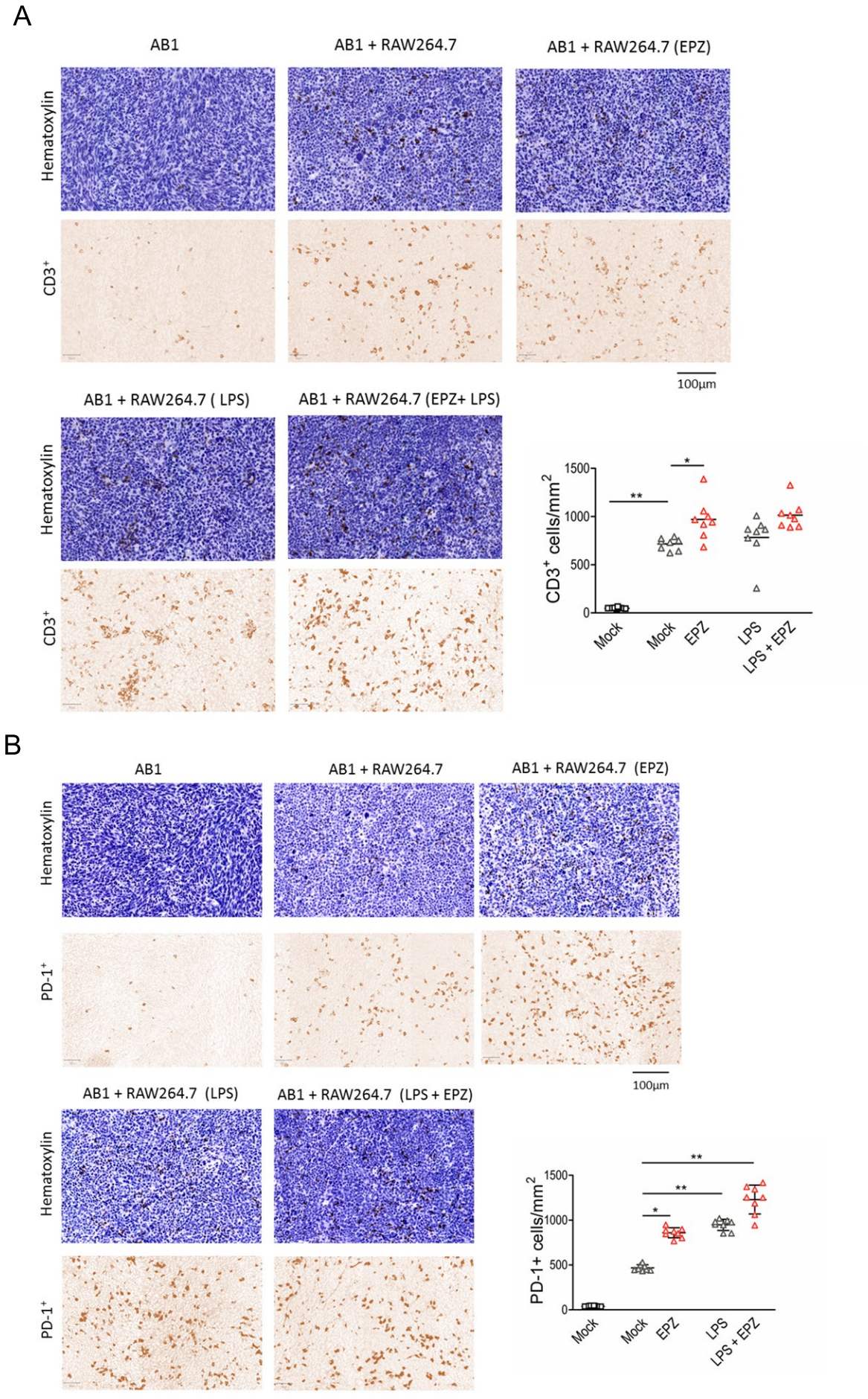
A
AB1
AB1 + RAW264.7
AB1 + RAW264.7 (EPZ)
Hematoxylin
CD3+
100μm
AB1 + RAW264.7 (LPS)
AB1 + RAW264.7 (EPZ+ LPS)
Hematoxylin
CD3+
CD3+ cells/mm2
1500
1000
500
0
**
*
Mock
Mock
EPZ
LPS
LPS + EPZ
B
AB1
AB1 + RAW264.7
AB1 + RAW264.7 (EPZ)
Hematoxylin
PD-1+
100μm
AB1 + RAW264.7 (LPS)
AB1 + RAW264.7 (LPS + EPZ)
Hematoxylin
PD-1+
PD-1+ cells/mm2
1500
1000
500
0
**
**
*
Mock
Mock
EPZ
LPS
LPS + EPZ

Figure S5: Immunohistochemistry of CD3 and PD-1 in AB1 mesothelioma tumors

BALB/c mice were inoculated with AB1 cells and RAW264.7 macrophages differentiated in presence or absence of EPZ and/or LPS. Tumor sections were labeled with (**A**) anti-CD3 (ThermoFischer) or **(B)** anti-PD-1 (Cell Signaling) monoclonal antibodies. After incubation with a HRP conjugate, sections were stained with hematoxylin. In the bar graph, the number of CD3+ cells per mm^2 in the central area of the tumor (i.e. 1 mm from the border) was determined using Image J and QuPath software. Statistical significance (*) at $p < 0.05$ was evaluated using Friedman test followed by Dunn's multiple comparison test.

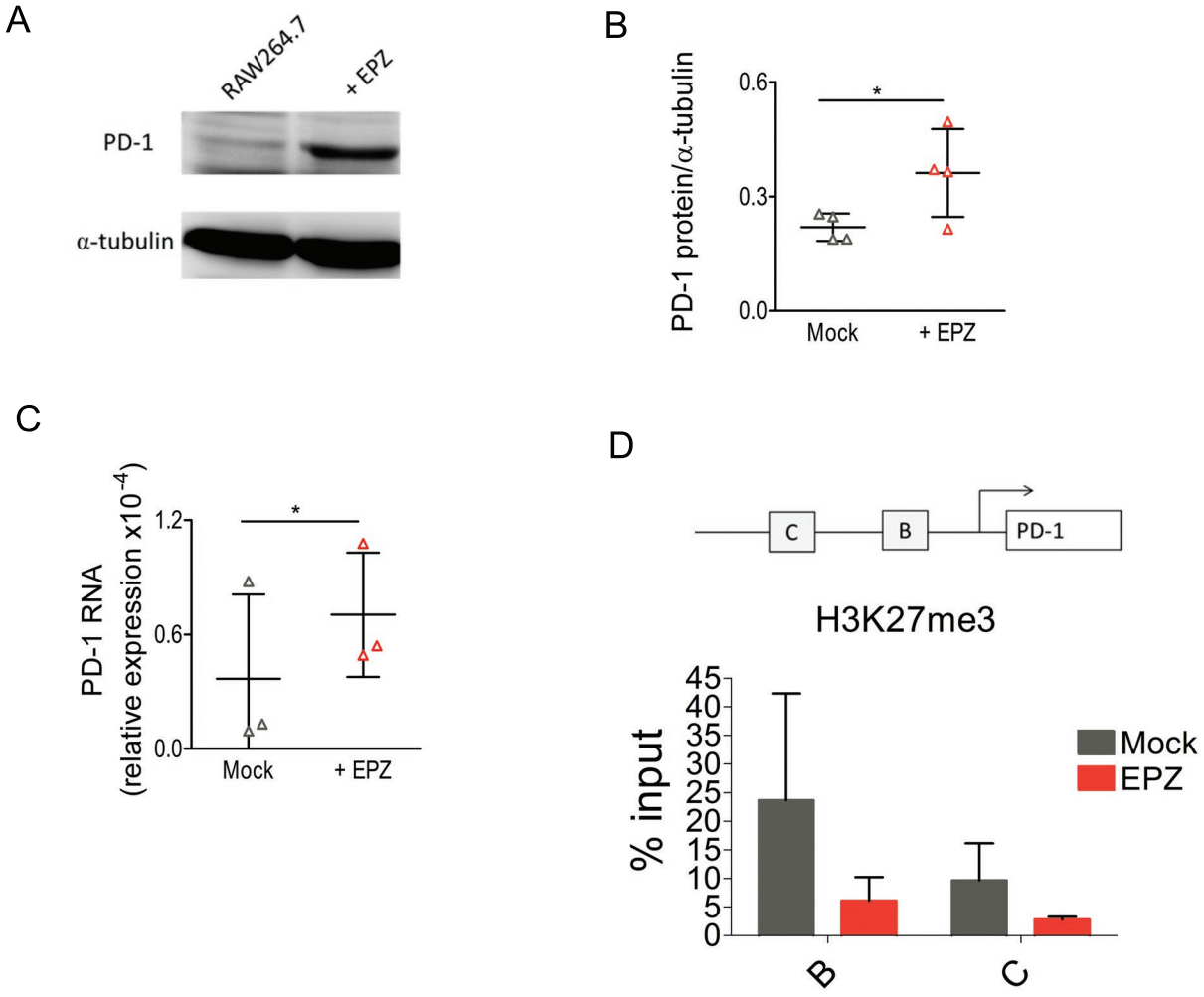

Figure S6: Effect of EPZ on PD-1 expression and H3K27me3 status at the PD-1 promoter

RAW264.7 macrophages were cultured in presence of LPS and/or EPZ as described in Figure 3. **(A)** RAW264.7 cell lysates were separated by SDS-PAGE, transferred onto nitrocellulose membranes and incubated with anti-αtubulin (Sigma) or anti-PD1 (Cell Signaling) antibodies. After incubation with corresponding HRP conjugates, the immunoblots were revealed using enhanced chemiluminescence (ECL, Pierce). (**B**) Band intensities were quantified with Image J software. **(C)** Total RNA was prepared from whole cells using NucleoSpin RNA Plus Kit (Macherey-Nagel) according manufacturer's instructions. After reverse transcription using FastGene Scriptase II cDNA Kit (Nippon Genetics), the abundance of transcripts was assessed by qPCR using the Takyon SYBR MasterMix (Eurogentec) and primers specific for PD-1 (5'- CGTCCCTCAGTCAAGAGGAG-3' ; 5'-GTCCCTAGAAGTGCCCAACA-3') and 18S (5'- GTAACCCGTTGAACCCCATT-3' ; 5'-CCATCCAATCGGTAGTAGCCG-3'). Samples were amplified in triplicate on each plate and data were analyzed using LightCycler 480 Software (Roche Diagnostics). Normalization was performed using mouse 18S as an internal control and relative gene expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ method. **(D)** Chromatin immunoprecipitation of the PD-1 promoter (regions B and C) using anti-H3K27me3 antibody. Assays were performed using the iDeal ChIP for histones (Diagenode).

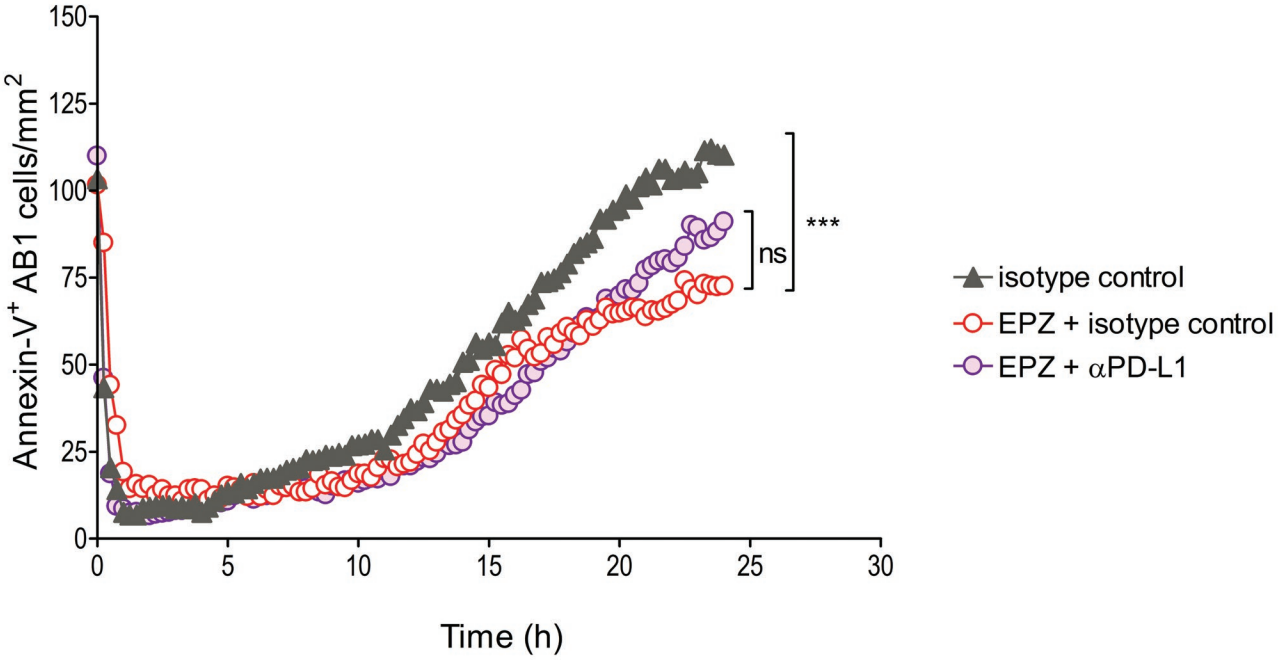

Figure S7: Effect of anti-PD-L1 blockade on AB1/RAW264.7 cocultures

RAW264.7 cultured with EPZ during 48 hours were co-cultivated with CFSE-labeled AB1 cells in presence of annexin V-APC and anti-PD-L1 antibody (10µg/mL InVivo Mab; Bioxcell) or rat IgG2b isotype control. The cells were monitored by the IncuCyte S3 Live-Cell imaging system (Essen Bioscience) placed in an incubator maintained at 37°C in a humidified 5% CO_2 atmosphere. The number of annexin-V-positive AB1 cells (%) was quantified each 15 min during 24h. Statistical significance was evaluated two-way ANOVA with Bonferroni posttest. *** means highly statistically significant ($p < 0.001$).

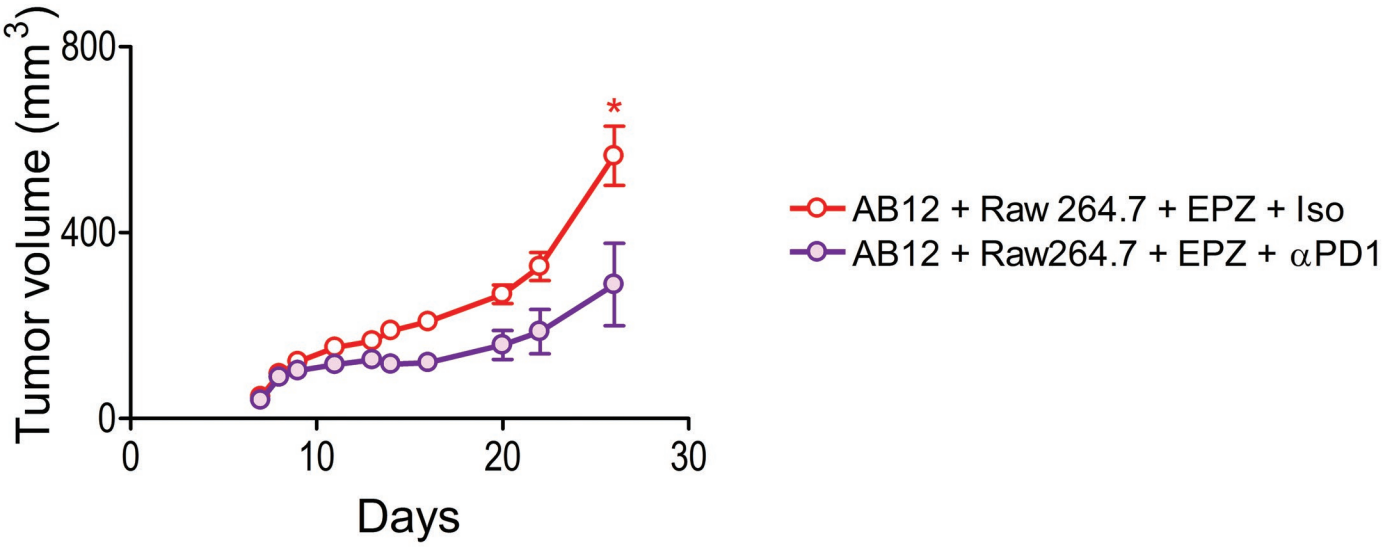

Figure S8: Effect of PD-1 blockade on AB12+RAW264.7 tumor growth

RAW264.7 macrophages were cultured with EZH2 inhibitor (10µM EPZ5687) for 24 hours and with anti-PD1 antibody (10µg/mL InVivo Mab, Bioxcell) or rat IgG2a isotype control (10µg/mL, Becton Dickinson). After 6 hours, RAW264.7 were washed and implanted subcutaneously in BALB/c mice together with 2.10^6 AB12 cells at a 1:3 ratio. Tumor volumes (in mm^3) were calculated weekly using the formula: $4/3 \times \pi \times (\text{diameter}/2)^3$. Groups of at least 6 mice were tested in each experimental condition. All data are plotted as means +/- SEM (n = 6). Statistical significance was evaluated using two-way ANOVA with Bonferroni posttest. (*), (**) and (***) mean statistically significant ($p < 0.05$), very statistically significant ($p < 0.01$) and highly statistically significant ($p < 0.001$), respectively.